

Combining Ability of Binary Mixtures of Native, Warm-Season Grasses and Legumes

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ABSTRACT

Growing complementary plant species is an alternative approach to enhancing pasture production. Our objective was to estimate combining ability for native, warm-season grasses and legumes grown in binary mixtures in the field using a combining ability analysis of variance. Six monocultures and 15 binary mixtures of the following species were studied: big bluestem, *Andropogon gerardii* Vit.; Illinois bundleflower, *Desmanthus illinoensis* (Michx.) MacM.; roundhead lespedeza, *Lepedeza capitata* Michx.; slender lespedeza, *L. virginica* (L.) Britt.; switchgrass, *Panicum virgatum* L.; and indiangrass, *Sorghastrum nutans* (L.) Nash. General combining ability (GCA) effects were found for forage dry matter yields (DMY, $P \leq 0.05$) of Illinois bundleflower (-1240 kg ha^{-1}), roundhead lespedeza (-3460 kg ha^{-1}), slender lespedeza (-3300 kg ha^{-1}), and switchgrass (8370 kg ha^{-1}). Specific combining ability (SCA) effects were found for DMY ($P \leq 0.1$) of switchgrass-legume mixtures (1360 kg ha^{-1}) and indiangrass-Illinois bundleflower mixtures (1230 kg ha^{-1}). General combining ability and SCA effects were found for crude protein concentration (CPC) of all species and mixtures ($P \leq 0.1$), respectively. General combining ability effects were found for in vitro dry matter digestibility (IVDMD) for switchgrass and the three legume species ($P \leq 0.05$). The compatibility of these species could not be predicted solely by DMYs. Compatible mixtures, however, were identified with greater confidence when other variables, such as CPC, IVDMD, and visual observations, were taken into account. On the basis of total forage protein (DMY times CPC), the only compatible grass-legume mixture was indiangrass-Illinois bundleflower (SCA effect = 100 kg ha^{-1} , $P \leq 0.05$).

MANY PRODUCERS use or want to adopt native, warm-season grasses for pasture. In the southern Great Plains, native, warm-season grasses begin growth in early to mid-April and provide excellent forage until the end of June. On rangelands consisting primarily of big bluestem, cattle weight gains of $1.0 \text{ kg head}^{-1} \text{ d}^{-1}$ from April to June are common (Owensby and Anderson, 1967). As plants form reproductive tillers, generally in July or August, forage becomes less palatable and nutritious; consequently, animal gains decline. By October, dietary supplements are needed to avoid weight loss. One approach to enhance pasture forage production and maintain quality is to over-seed grass pastures with one or more forage legume species. Forage legumes provide a renewable source of nitrogen for plant growth and quality forage for grazing livestock (Hoveland, 1989).

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Published in Crop Sci. 41:818–823 (2001).

Legumes may also be used to extend the production season in the cool- and warm-season grass pastures.

Grazing systems using forage legumes increase animal production (Fribourg et al., 1979; Jung et al., 1985; Rayburn et al., 1980; Stricker et al., 1979), and pastures with legumes have greater crude protein content, digestibility, and mineral composition for livestock diets, resulting in greater forage intake and animal performance (Marten, 1985). Kroth et al. (1982) reported the nitrogen (N) benefits from birdsfoot trefoil (*Lotus corniculatus* L.) and alfalfa (*Medicago sativa* L.), producing 115 and 200 kg N ha^{-1} , respectively, annually. Residual N fixed by legumes increased subsequent forage growth of ryegrass (*Lolium multiflorum* Lam.) and was equivalent to a fertilization with 111 kg N ha^{-1} for arrowleaf clover (*Trifolium vesiculosum* Savi) and 121 kg N ha^{-1} for a mixture of arrowleaf and crimson clovers (*Trifolium incarnatum* L.; Lynd et al., 1984).

Legumes and grasses grown in mixtures can either be compatible—avoid competition with each other, competitive—make demands on the same resources, or allelopathic—interact with each other (Harper, 1977). These relationships are difficult to measure in traditional plot and grazing experiments because dominant species in mixtures have competitive advantages at the onset of measuring their compatibility and interactions. Combining abilities for species and for specific species mixtures can be estimated by a combining ability analysis of variance. This analysis has typically been used by plant and animal breeders to estimate combining abilities of breeding lines using variance components (Griffing, 1956). We used this approach to estimate combining abilities of binary mixtures of grasses and legumes (Springer et al., 1996).

Producers need information concerning combining abilities for cool- and warm-season species presently grown or having the potential of being grown for forage. In other regions, laboratory, greenhouse, and field studies have shown allelopathic and competitive effects of tall fescue (*Festuca arundinacea* Schreb.) and white clover (*Trifolium repens* L.) toward other species (McCloud and Mott, 1953; Peters, 1968; MacFarlane et al., 1982; Springer et al., 1996; Springer, 1996). Compatibility has been shown for tall fescue with either birdsfoot trefoil or white clover (Pederson and Brink, 1988; Beuselinck et al., 1992; Springer et al., 1996) and for switchgrass, indiangrass, or sideoats grama (*Bouteloua curtipendula* Michx.) mixed with either purple prairieclover [*Petalostemon purpureum* (Vent.) Rydb.], roundhead lespedeza, leadplant (*Amorpha canescens* Pursh), Illinois bundleflower, catclaw sensitive brier [*Schrankia nuttallii*

Abbreviations: CPC, crude protein concentration; DM, dry matter; DMY, dry matter yield; GCA, general combining ability; IVDMD, in vitro dry matter digestibility; SCA, specific combining ability.

(DC.) Standl.], or cicer milkvetch (*Astragalus cicer* L., Posler et al., 1993).

Little information is available regarding the combining ability of warm-season grasses and legumes. Much of the combining ability literature is from laboratory and greenhouse experiments using cool-season forage species. Our objective was to estimate the combining ability effects for native, warm-season grasses and legumes grown in binary mixtures in the field.

MATERIAL AND METHODS

This study was conducted at the USDA-Agricultural Research Service, Dale Bumpers Small Farms Research Center, near Booneville, AR, (35°06' N, 93°54' W) on a Leadvale (fine-silty, siliceous, thermic Typic Fragiudults) soil. Soil tests of the experimental site showed the soil nutrient content of 10 kg ha⁻¹ NO₃-N, 43 kg ha⁻¹ NH₄-N, 19 kg ha⁻¹ phosphorus (P), and 135 kg ha⁻¹ potassium (K). The soil pH value was 5.0. In February 1995, 72 kg ha⁻¹ N, 72 kg ha⁻¹ P, 72 kg ha⁻¹ K, and 2.5 Mg ha⁻¹ of lime were incorporated into the soil using a disk (16 cm). The source of the nutrients was a blended fertilizer (13-13-13, N-P-K) and dolomitic limestone. Before transplanting, the plot area was disked to kill weeds and leveled with a harrow.

The species studied were 'Kaw' big bluestem, 'Alamo' switchgrass, 'Osage' indiangrass, 'Sabine' Illinois bundleflower, 'Kanoka' roundhead lespedeza, and common slender lespedeza. Seeds of each species were germinated and grown in cavity seedling trays (196 cavities tray⁻¹) and maintained in a greenhouse before field planting. Legume seeds were inoculated with specific *Rhizobium* before germination.

In April 1995, each species was transplanted by hand into field plots on 15-cm centers. Mixtures were planted at a 1:1 ratio alternating species within and between rows. Treatments consisted of the six pure stands and fifteen mixed stands. All combinations of species mixtures were used, i.e., grass with grass, grass with legume, and legume with legume. The field plot design was a randomized complete block replicated four times with individual plots 1.2 by 1.4 m in size. During the establishment year, plots were maintained weed-free by hoeing and dead plants were replaced to maintain plant populations.

In early March 1996 and 1997, before the initiation of new growth, residual dry-matter (DM) was removed from plots by burning. In mid-March each year, a broadcast fertilization of P and K was applied at a rate of 67 kg ha⁻¹ to all plots. The source of P was triple superphosphate (0-46-0, N-P-K) and the source of K was potash (0-0-60, N-P-K). At the same time, grass-only plots received 67 kg ha⁻¹ of N as ammonium nitrate (34-0-0, N-P-K).

Plots were harvested in the last week of June 1996 and 1997. Grasses were in the boot (R0) to early inflorescence emergence (R1) stage of growth at harvest (Moore et al., 1991). The forage DMY of each plot was determined by harvesting the entire plot to a stubble height of 10 cm. The plot was weighed fresh and a 250 to 300 g subsample of forage was collected for DM determination. The forage of each plot was then separated by hand into its respective species components. Each component was weighed fresh and a 250 to 300 g subsample of forage was collected for DM determination. Forage sub-samples were oven dried at 60°C. The DMY of each plot was calculated by multiplying the percentage DM of the oven dried subsample by the harvested green weight of the plot and converted to kilograms per hectare. The percentage of each species in mixture was calculated by dividing its DM

weight by the total DM weight of the plot. Oven dried subsamples were ground to pass a 1-mm screen. Crude protein (N% × 6.25) was determined by the Micro-Kjeldahl procedure (AOAC, 1997) and IVDMD was determined by the Goering and Van Soest (1970) procedure modified to digest samples in an ANKOM Daisy II *In Vitro* Digester (ANKOM Technology Corp., Fairport, NY).

The lack of significant regrowth due to rainfall deficits after the June harvest each year precluded another harvest. The long-term average rainfall for the site is 10.6 cm in July and 7.7 cm in August. In 1996, rainfall was 4.0 cm above average in July and 4.0 cm below average in August. Although above average rainfall fell in July 1996, 36% was recorded in the second week of July and 59% in the fourth week of July. In 1997, rainfall was 8.3 cm below average in July and 2.0 cm below average during August.

Data for DMY, CPC, IVDMD, total forage protein (DMY times CPC), and total digestible forage (DMY times IVDMD) were analyzed as a split-plot in time analysis of variance with species mixture (treatment) as the main plot and year as the subplot (SAS Institute, 1988). Data were analyzed separately for each year when year effects or year × treatment interactions were present ($P \leq 0.05$). Comparisons of means were made by Fisher's (protected) least significant difference (LSD) at $P \leq 0.05$ (Steel and Torrie, 1980).

The combining abilities of species and species mixtures were determined by a combining ability analysis of variance (Griffing, 1956) Method 4, Model 1 procedure and PROC GLM (SAS Institute, 1988). By definition, the general combining ability (GCA) is the mean performance of a species when expressed as a deviation from the overall mean of all species combinations. The specific combining ability (SCA) is the deviation of the "expected" value (the overall mean plus the sum of the GCAs of the two species in mixture) from the mean value of the two species in mixture. Similar to the concept of "Relative Yield Total" as summarized by Harper (1977), we defined an SCA effect = 0 to indicate a competition between species, e.g., the species make similar demands on resources. When the SCA effect = 0, each species contribution to the mixture is equal to its expected share. We defined an SCA effect > 0 to denote a compatibility between species, e.g., the species avoided competition by making different demands on resources. When SCA effect > 0, each species contribution to the mixture is greater than its expected share. An SCA effect < 0 suggested an incompatibility between species, e.g., the species interact with each other. When SCA effect < 0, each species contribution to the mixture is less than its expected share.

RESULTS AND DISCUSSION

Variation due to mixtures and year × mixture interactions were found for DMY, CPC, and IVDMD ($P \leq 0.05$). Dry-matter yields of grass mixtures were generally three to four times greater than legume mixtures and grass-legume mixtures were generally equal to or greater than legume mixtures (Table 1). In addition, DMYs of grass-legume mixtures were equal to or slightly greater than their corresponding grasses grown in pure stands ($P \geq 0.05$). This last result differs from Posler et al. (1993), who reported substantially greater forage yields for 17 of 18 grass-legume mixtures compared to the corresponding grasses grown alone. Four of nine species used in their study were used in our study. The difference between our experiments is possibly due

Table 1. Dry matter yield (DMY), crude protein concentration (CPC), and in vitro digestion (IVDMD) for mixtures and pure stands of warm-season grasses and legumes in 1996 and 1997.

Species	DMY		CPC		IVDMD	
	1996	1997	1996	1997	1996	1997
	— kg ha ⁻¹ —		— g kg ⁻¹ —			
Mixtures†						
Grass-grass						
BB SG	19 430	18 680	45	41	519	520
BB IG	13 370	9 630	51	47	577	545
SG IG	17 900	17 400	48	44	495	519
Grass-legume						
BB IB	10 470	11 650	87	62	577	518
SG IB	21 050	20 360	54	54	495	523
IG IB	11 200	12 750	74	94	559	492
BB RL	8 790	10 000	69	49	548	550
SG RL	20 810	16 300	44	40	494	518
IG RL	10 940	8 430	55	45	582	549
BB SL	8 840	10 050	63	50	563	589
SG SL	19 560	17 600	47	43	515	515
IG SL	10 710	7 940	49	46	566	553
Legume-legume						
IB RL	5 430	6 060	147	141	590	572
IB SL	5 160	8 160	156	128	612	555
RL SL	5 000	2 750	121	97	523	598
Pure stands						
BB	12 760	9 850	60	56	584	582
SG	17 790	19 250	49	38	504	500
IG	11 170	6 920	53	44	556	561
IB	5 450	8 060	160	127	604	511
RL	4 670	3 400	127	109	507	534
SL	2 460	2 970	118	89	513	580
LSD _(0.05)	3 790	3 410	13	17	44	51

† BB, big bluestem; SG, switchgrass; IG, indiangrass; IB, Illinois bundleflower; RL, roundhead lespedeza; SL, slender lespedeza.

to soil fertility. The soil of their experiment site was low in available NO₃-N. The soil of our site was amended with NO₃-N before planting and a maintenance fertilization of NO₃-N was added to grass plots each year.

Crude protein concentrations averaged 122 g kg⁻¹ for legume pure stands, 132 g kg⁻¹ for legume mixtures, 57 g kg⁻¹ for grass-legume mixtures, 46 g kg⁻¹ for grass mixtures, and 50 g kg⁻¹ for grass pure stands (Table 1). Crude protein concentrations averaged 90 g kg⁻¹ for grass-legume mixtures and 38 g kg⁻¹ for grass pure stands in the study by Posler et al. (1996). Grass-legume mixtures in our study had equal plant populations (50% grass, 50% legume) because plots were planted by hand. Grass-legume mixtures in their study had unequal plant populations because plots were seeded directly. Greater contributions of legumes in DMY of mixtures could account for the greater CPC found in grass-legume mixtures in their study. Crude protein concentrations for grass pure stands in our study were greater probably because grass plots were fertilized with NO₃-N each year.

In vitro digestion averaged 542 g kg⁻¹ for legume pure stands, 575 g kg⁻¹ for legume mixtures, 539 g kg⁻¹ for grass-legume mixtures, 529 g kg⁻¹ for grass mixtures, and 548 g kg⁻¹ for grass pure stands (Table 1). In vitro digestion averaged 480 g kg⁻¹ for grass-legume mixtures and 475 g kg⁻¹ for grass pure stands in the study by Posler et al. (1996). Although our overall data are 50 to 100 g kg⁻¹ greater in IVDMD, neither study found appreciable differences between the digestibilities of grass-legume mixtures and grasses in pure stands.

Table 2. The average species composition of each mixture in 1996 and 1997 (percentages based on above ground dry-matter).

Species mixture†	First species in mixture		Second species in mixture	
	1996	1997	1996	1997
	— % —			
Grass-grass				
BB SG	39	30	61	70
BB IG	51	64	49	36
SG IG	63	80	37	20
Grass-legume				
BB IB	70	54	30	46
SG IB	79	72	21	28
IG IB	68	49	32	51
BB RL	76	74	24	26
SG RL	84	86	16	14
IG RL	76	59	24	41
BB SL	90	80	10	20
SG SL	93	89	7	11
IG SL	89	66	11	34
Legume-legume				
IB RL	58	65	42	35
IB SL	79	71	21	29
RL SL	73	53	27	47

† BB, big bluestem; SG, switchgrass; IG, indiangrass; IB, Illinois bundleflower; RL, roundhead lespedeza; SL, slender lespedeza.

A year × GCA interaction for DMY is explained by the increase in GCA for Illinois bundleflower from -2,400 to -70 kg ha⁻¹ ($P \leq 0.01$). The proportion of Illinois bundleflower in mixtures increased from 1996 to 1997 (Table 2). This increase was from increases in plant size as well as seedling recruitment. The harvest height of 10 cm allowed for some flowering and seed production on the lowest branches of Illinois bundleflower. The year × GCA interaction for CPC resulted from magnitude differences among species ($P \leq 0.01$). The trends from 1996 to 1997 were the same, where grass species had positive GCAs for DMY and negative GCAs for forage quality and legumes had negative GCAs for DMY and positive GCAs for forage quality (Table 3). The year × GCA interaction for IVDMD was probably caused by the decrease in IVDMD for Illinois bundleflower and increases in IVDMD for roundhead lespedeza and slender lespedeza in 1996 compared to 1997 ($P \leq 0.01$, see Table 1 under “Pure stands”).

Specific combining ability effects for DMY were found for switchgrass-legume mixtures ($P \leq 0.1$) and indiangrass-Illinois bundleflower mixtures ($P \leq 0.1$, Table 4). Posler et al. (1993) observed that Illinois bundleflower was quite competitive with switchgrass and indiangrass. The data in our experiment, analyzed with the combining ability analysis, suggested that Illinois bundleflower was compatible with both grass species ($P \leq 0.1$). The earlier discussion on differences in plant populations between the two experiments and the fact that Illinois bundleflower has excellent seedling vigor, which may give it a competitive advantage when direct seeded with these grasses, may explain the different conclusions.

Specific combining ability effects for CPC were found for all species combinations ($P \leq 0.01$ for all species combination except indiangrass-Illinois bundleflower

Table 3. General combining ability effects for dry matter yield (DMY), crude protein concentration (CPC), in vitro digestion (IVDMD), total forage protein (TFP), and total digestible forage (TDF) for mixtures of warm-season grasses and legumes grown in 1996 and 1997. The year means for each variable are given below the general combining ability effects for that year and the overall mean for each variable is given at the bottom of the table.

Year	Species†	DMY	CPC	IVDMD	TFP	TDF
		— kg ha ⁻¹ —	— g kg ⁻¹ —		— kg ha ⁻¹ —	
1996	BB	-500	-14***	11	-60	20
	SG	8 970***	-33***	-55***	210***	4 010***
	IG	320	-23***	10	-100***	400
	IB	-2 400***	37***	24***	160***	-1 150***
	RL	-2 980***	16***	0	-80	-1 580***
	SL	-3 410***	17***	10	-120***	-1 710***
1996 mean		12 578	74	548	772	6 737
1997	BB	190	-19***	4	-120***	250
	SG	7 770***	-26***	-28***	140***	3 840***
	IG	-780	-13***	-12	-70*	-490*
	IB	-70	38***	-12	360***	-160
	RL	-3 930***	11***	21***	-190***	-1 930***
	SL	-3 190***	9***	26***	-130***	-1 510***
1997 mean		11 848	65	541	680	6 290
Overall mean		12 213	70	544	726	6 514

Significantly different from zero (0) at $P \leq 0.1^*$, 0.05^{**} , and 0.01^{***} .

† BB, big bluestem; SG, switchgrass; IG, indiangrass; IB, Illinois bundleflower; RL, roundhead lespedeza; SL, slender lespedeza.

where it was $P \leq 0.09$) and were positive for mixtures of grasses or legumes and negative for grass-legume mixtures (Table 4). The negative SCAs for the grass-legume mixtures can be explained by unequal contributions of forage in the samples. In grass-legume mixtures, the forage was dominated by the grass component. The mixture of switchgrass-Illinois bundleflower averaged 76% grass and 24% legume (Table 2). This resulted in lower CPCs than would be expected if the sample were equally represented by both components in the mixture. For indiangrass-Illinois bundleflower, the components were more equally represented in the mixture, averaging 59% indiangrass versus about 41% Illinois bundleflower (Table 2).

For calculated total forage protein (DMY times CPC), the only significant SCA effect was indiangrass-Illinois bundleflower (SCA effect = 100 g kg⁻¹ crude

protein, $P \leq 0.05$). As stated earlier, those species combinations with significantly high SCA were considered compatible with each other. The SCA effect for this mixture could be attributed to its higher CPC and higher than expected DMY. On the basis of partial correlation coefficients, CPC was more closely associated with total forage protein of indiangrass-Illinois bundleflower (partial $R^2 = 0.67$, $P < 0.01$) than was DMY (partial $R^2 = 0.32$, $P < 0.01$).

Year \times SCA effects ($P \leq 0.02$) were found only for IVDMD. Several factors could be involved in this interaction. The most obvious are the differences in digestibilities among the pure stands of legumes as discussed earlier for year \times GCA interactions. Overall, no trend was observed for SCA effects for IVDMD and the only significant grass-legume mixture was indiangrass and Illinois bundleflower (-24 g kg⁻¹, Table 4).

Table 4. Specific combining ability effects for dry matter yield (DMY), crude protein concentration (CPC), in vitro digestion (IVDMD), total forage protein (TFP), and total digestible forage (TDF) for mixtures of warm-season grasses and legumes grown in 1996 and 1997. The overall mean for each variable is given at the bottom of the table.

Species mixture†	DMY	CPC	IVDMD	TFP	TDF
	— kg ha ⁻¹ —	— g kg ⁻¹ —		— kg ha ⁻¹ —	
Grass-grass					
BB SG	-1 380*	20***	9	3	-650
BB IG	-330	14***	10	14	-100
SG IG	-2 700***	24***	5	-20	-1 510***
Grass-legume					
BB IB	230	-16***	-11	-75	70
SG IB	1 350*	-24***	0	-56	730*
IG IB	1 230*	-5*	-24**	100**	450
BB RL	790	-8***	-13	50	290
SG RL	1 430*	-12***	-7	26	710*
IG RL	1 160	-16***	12	-19	800**
BB SL	680	-10***	6	8	390
SG SL	1 300*	-8***	-6	47	730**
IG SL	640	-17***	-2	-76	350
Legume-legume					
IB RL	-1 780**	23***	21**	-24	-780**
IB SL	-1 030	22***	15	54	-460
RL SL	-1 590**	12***	-12	-33	-1 020***
Overall mean	12 213	70	544	726	6 514

Significantly different from zero (0) at $P \leq 0.1^*$, 0.05^{**} , and 0.01^{***} .

† BB, big bluestem; SG, switchgrass; IG, indiangrass; IB, Illinois bundleflower; RL, roundhead lespedeza; SL, slender lespedeza.

However, four grass-legume combinations show significant SCA effects for calculated total digestible forage (DMY times IVDMD, Table 4). We could attribute the SCA effects for these mixtures to higher than expected DMYs. On the basis of partial correlation coefficients, DMY was more closely associated with total digestible forage of the four grass-legume mixtures (partial $R^2 = 0.97$, $P < 0.01$) than was IVDMD (partial $R^2 = 0.02$, $P < 0.01$).

CONCLUSIONS

Each of the grass-legume mixtures in this study is useful for forage. Their long-term usefulness, however, depends on their ability to persist in mixture. The majority of the grass-legume mixtures showed some form of competition. One species is eventually favored over the other when competition occurs, thus leading to a lack of persistence for the eliminated species. The time it takes for one species to out-compete another species was not in the scope of this experiment; however, competition will eventually lead to renovation of the pasture if the mixture is to continue. Compatible mixtures in this study were also useful for forage. These mixtures somehow avoid competition. The characteristics used by these species to avoid competition are unknown and deserve further research.

Cultivar differences were not addressed in this experiment. In a similar experiment, Springer et al. (1996) found differences in combining ability between tall fescue cultivars and legume species. However, cultivars with similar gross morphology and growth habits to these species should give similar results.

Selective grazing by livestock was not addressed in this experiment. For example, preferential grazing of legumes may reduce the legume component in mixtures with grasses. Unless the legume is tolerant of grazing, it may not persist in mixtures.

In our experiment, the compatibility of these species could not be predicted solely on DMYs. Compatible mixtures, however, were identified with greater confidence when other variables, such as crude protein concentration, in vitro digestibility, and visual observations, were taken into account. Under the conditions of our experiment, a mixture of indiangrass-Illinois bundleflower was compatible for total forage protein. This species combination should persist well under closely managed grazing because the legume has good seed set and seedling recruitment. Although legume mixtures with Alamo switchgrass did show some promise in the experiment, visual observations suggested that over time Alamo switchgrass may shade out these legume species. Switchgrass accounted for 84% of the DM for switchgrass-legume mixtures.

Producers need information about the ability of cool- and warm-season species to coexist and thrive together. In this experiment, a mixture of indiangrass-Illinois bundleflower was the most compatible grass-legume mixture as determined on the basis of total forage protein (SCA effect = 100, $P \leq 0.05$), while a mixture of big bluestem-Illinois bundleflower was incompatible (SCA

effect = -70, $P \leq 0.15$). Providing information on specific combining abilities for species mixtures would allow producers to choose species for their forage program.

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CELL BIOLOGY & MOLECULAR GENETICS

A Genetic Linkage Map of Diploid *Paspalum notatum*

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ABSTRACT

Paspalum notatum Flugge is a subtropical grass native to South America. The most common form in the USA is *P. notatum* var. *saurae* Parodi (Pensacola bahiagrass), which is a valuable forage. Pensacola bahiagrass is a sexual diploid, while most other races of *P. notatum* are apomictic tetraploids. The objective of this work was the construction of a genetic linkage map of diploid *P. notatum* ($2n = 2x = 20$) that can be used as a framework for basic genetic studies as well as breeding purposes. The mapping population derived from a cross between the genotypes Q4084₁₀ and Tift₉ that originated from Cayastá, Santa Fe, Argentina, and Tifton, GA, USA, respectively. Heterologous restriction fragment length polymorphism (RFLP) clones of maize (*Zea mays* L.), rice (*Oryza sativa* L.), and oat (*Avena sativa* L.) were used to cover the *Paspalum* genome uniformly in a comparative approach, while random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers were added to condense the linkage groups. A combined map was constructed with the markers segregating from both parental genotypes by the program JoinMap 1.4. A total of 149 marker loci were used for map construction. One hundred twelve loci were allocated to 10 linkage groups, covering a total map distance of 991 centimorgan (cM). The average distance between markers was about 9 cM. *Paspalum* Linkage Groups 1, 3, 4, 5, 6, 8, and 10 showed syntenic regions with maps of maize and rice. Moreover, several RFLP loci reported to be associated with apomixis in hybrids of maize-*Tripsacum* and *Brachiaria* were located on the map. This study provides a genetic linkage map of a subtropical forage grass with both sexual and apomictic forms, which can be used for investigating simple and complex traits.

PASPALUM is a large genus of the grass family consisting of more than 400 species, most of which are native to the tropical and subtropical regions of the New

World (Chase, 1929). Several members of the genus are of economic importance since they are used for forage, turf, and ornamental purposes (Burson and Bennett, 1971). Approximately 80% of the species are polyploid, and among them, tetraploids are predominant (Quarín, 1992). Apomixis (i.e., asexual reproduction by seeds) is a common trait in the genus, being apospory (in which a non reduced megagametophyte originates from a somatic cell, usually a nucellar cell) the most frequent type, and diplospory (in which a non reduced megaspore originates from the reproductive cell itself, with the later failing to successfully complete meiosis) only of occasional occurrence (Quarín, 1992). As in other grasses, apomixis takes place in *Paspalum* mainly at the polyploid level. However, diploids with some degree of apomictic reproduction have been observed (Norrman et al., 1989). In nature, apomictic tetraploids usually appear together with sexual diploid cospecific races.

Paspalum notatum Flugge is a rhizomatous species widely distributed in native grasslands from central Mexico to Argentina and throughout the Caribbean Islands (Chase, 1929). The diploid form, Pensacola bahiagrass (*P. notatum* var. *saurae* Parodi, $2n = 2x = 20$), is one of the most popular perennial pasture grasses in the southern USA (Burton, 1974). Although it was named after the Pensacola region of Florida, it probably originated from a relatively small area of central-eastern and northeastern Argentina (Burton, 1967). The species is sexual and self-incompatible (Burton, 1955).

Molecular marker technologies have led to the rapid development of detailed genetic maps for many crop plants (Martin, 1998). Genetic linkage maps provide a framework for studying simple and complex traits. Moreover, the possibility of using common RFLP probes across several taxa provides a means of comparing maps between both related and unrelated species (Ahn and Tanksley, 1993). Comparative studies in the Gramineae have led to the observation of an extensive similarity between genomes of several species (Devos and Gale, 1997; Glaszmann et al., 1997; Moore et al., 1995). With regard to the forage grasses, genetic linkage maps are available for *Lolium*, where a number of im-

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Abbreviations: AFLP, amplified fragment length polymorphism; cM, centimorgan; LOD logarithms of the odds; PCR, polymerase chain reaction; QTL, quantitative trait loci; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism.